

Attorney Docket No.: 44158/244344 (SJ-0029)
Inventors: Schuetz et al.
Serial No.: 09/974,619
Filing Date: October 10, 2001
Page 24

This listing of the claims will replace all prior versions and listings of claims in the application:

Listing of the claims:

Claim 1: (currently amended) A method for predicting CYP3A5 expression level in a subject comprising determining the nucleotide present in each CYP3A5 allele of the genomic DNA of said subject at the location(s) selected from the group consisting of:

(a) the position corresponding to nucleotide 23 of SEQ ID NO:73 within intron 3 of the Cyp3A5 gene:

(b) the position corresponding to nucleotide 29 of SEQ ID NO:74 within exon 7 of the Cyp3A5 gene; and

(c) the positions corresponding to both nucleotide 23 of SEQ ID NO:73 and nucleotide 29 of SEQ ID NO:74;

wherein the presence of an A at the position corresponding to nucleotide 23 of SEQ ID NO:73 on at least one CYP3A5 allele of said subject predicts a relatively high level of expression of CYP3A5 as compared to the presence of a G at that position and the presence of a G at the position corresponding to nucleotide 23 of SEQ ID NO:73 on each CYP3A5 allele of said subject predicts a relatively low level of expression of CYP3A5 as compared to the presence of an A at that position;

Attorney Docket No.: 44158/244344 (SJ-0029)
Inventors: Schuetz et al.
Serial No.: 09/974,619
Filing Date: October 10, 2001
Page 25

wherein the presence of a G at the position corresponding to nucleotide 29 of SEQ ID NO:74 on at least one CYP3A5 allele of said subject predicts a relatively high level of expression of CYP3A5 as compared to the presence of an A at that position and the presence of an A at the position corresponding to nucleotide 29 of SEQ ID NO:74 on each CYP3A5 allele of said subject predicts a relatively low level of expression of CYP3A5 as compared to the presence of a G at that position; and

wherein the presence of an A at the position corresponding to nucleotide 23 of SEQ ID NO:73 and a G at the position corresponding to nucleotide 29 of SEQ ID NO:74 on at least one CYP3A5 allele of said subject predicts a relatively high level of expression of CYP3A5 as compared to the presence of a G at the position corresponding to nucleotide 23 of SEQ ID NO:73 and an A at the position corresponding to nucleotide 29 of SEQ ID NO:74 on at least one CYP3A5 allele of said subject and the presence of either a G at the position corresponding to nucleotide 23 of SEQ ID NO:73 or an A at the position corresponding to nucleotide 29 of SEQ ID NO:74 on each CYP3A5 allele of said subject predicts a relatively low level of expression of CYP3A5 as compared to the presence of either an A at the position corresponding to nucleotide 23 of SEQ ID NO:73 or a G at the position

Attorney Docket No.: 44158/244344 (SJ-0029)
Inventors: Schuetz et al.
Serial No.: 09/974,619
Filing Date: October 10, 2001
Page 26

corresponding to nucleotide 29 of SEQ ID NO:74 on each CYP3A5
allele of said subject.

Claim 2: (previously presented) The method of claim 1
wherein said location is the position corresponding to nucleotide
23 of SEQ ID NO:73 within intron 3 of the Cyp3A5 gene.

Claim 3: (previously presented) The method of claim 1
wherein said location is the position corresponding to nucleotide
29 of SEQ ID NO:74 within exon 7 of the Cyp3A5 gene.

Claim 4: (previously presented) The method of claim 1
wherein said locations are the positions corresponding to both
nucleotide 23 of SEQ ID NO:73 and nucleotide 29 of SEQ ID NO:74.

Claim 5: (previously presented) The method of claims 1, 2, 3
or 4 wherein the step of determining the nucleotide present in
each CYP3A5 allele of said subject at the selected location(s) is
accomplished by sequencing a region of the genomic DNA of said
subject which includes said location(s).

Claim 6: (previously presented) The method of claims 1, 2, 3

Attorney Docket No.: 44158/244344 (SJ-0029)
Inventors: Schuetz et al.
Serial No.: 09/974,619
Filing Date: October 10, 2001
Page 27

or 4 wherein the step of determining the nucleotide present in each Cyp3A5 allele of said subject at the selected location(s) is accomplished by

(a) amplifying a region of the genomic DNA of said subject which includes said location(s) to generate an amplified fragment, and

(b) treating the amplified fragment with a restriction enzyme in its corresponding restriction buffer to determine the identity of the nucleotide present at the selected location(s).

Claim 7: (previously presented) The method of claims 1, 2, 3 or 4 wherein the step of determining the nucleotide present in each Cyp3A5 allele of said subject at the selected location(s) is accomplished by

(a) amplifying a region of the genomic DNA of said subject which includes said location(s), and

(b) hybridizing the amplified region with probes specific for the selected location(s) wherein hybridization determines the identity of the nucleotide present at the selected location(s).

Claim 8: (previously presented) A method for determining the cytochrome P450 3A5 (CYP3A5) genotype and phenotype of an

Attorney Docket No.: 44158/244344 (SJ-0029)
Inventors: Schuetz et al.
Serial No.: 09/974,619
Filing Date: October 10, 2001
Page 28

individual comprising:

- (a) isolating nucleic acid from the individual;
- (b) amplifying a region of the cytochrome P450 3A5 (CYP3A5)

gene sequence selected from the group of:

- (i) intron 3 comprising the position corresponding to nucleotide 23 of SEQ ID NO:73;
 - (ii) exon 7 comprising the position corresponding to nucleotide 29 of SEQ ID NO:74; and
 - (iii) intron 3 comprising the position corresponding to nucleotide 23 of SEQ ID NO:73 and exon 7 comprising the position corresponding to nucleotide 29 of SEQ ID NO:74; and
- (c) sequencing the amplified region of step (b), thereby determining the cytochrome P450 3A5 (CYP3A5) genotype and phenotype of the individual.

Claim 9: (previously presented) The method of claim 8 wherein the intron 3 region of cytochrome P450 3A5 (CYP3A5) is amplified utilizing primers which amplify 5' and 3' of the position corresponding to nucleotide 23 of SEQ ID NO:73.

Claim 10: (currently amended) The method of claim 9 wherein the intron 3 region is amplified utilizing SEQ ID NO: 24 and 25

Attorney Docket No.: 44158/244344 (SJ-0029)
Inventors: Schuetz et al.
Serial No.: 09/974,619
Filing Date: October 10, 2001
Page 29

primers, or a fragment thereof which is at least ten bases long,
or SEQ ID NO: 26 and 27 primers, or a fragment thereof which is
at least ten bases long.

Claim 11: (previously presented) The method of claim 8
wherein the exon 7 region of cytochrome P450 3A5 (CYP3A5) is
amplified utilizing primers which amplify 5' and 3' of the
position corresponding to nucleotide 29 of SEQ ID NO:74.

Claim 12: (currently amended) The method of claim 11
wherein the exon 7 region is amplified utilizing SEQ ID NO: 30
and 16 primers, or a fragment thereof which is at least ten bases
long, or SEQ ID NO: 31 and 32 primers, or a fragment thereof
which is at least ten bases long.

Claim 13-22: (canceled)

Claim 23: (currently amended) A method for determining
cytochrome P450 3A5 (CYP3A5) genotype of a subject which
comprises

- (a) isolating nucleic acid from said subject;
- (b) amplifying a cytochrome P450 3A5 (CYP3A5) PCR fragment

Attorney Docket No.: 44158/244344 (SJ-0029)
Inventors: Schuetz et al.
Serial No.: 09/974,619
Filing Date: October 10, 2001
Page 30

from said nucleic acid using a set of primers, wherein said set of primers contains primer X and primer Y; wherein

~~(i) the X primer is complementary to a region 5' to the position corresponding to nucleotide 23 of SEQ ID NO:73; and~~

~~———— (ii) the Y primer is complementary to a region 3' to the position corresponding to nucleotide 23 of SEQ ID NO:73;~~

(i) primer X has the sequence of SEQ ID NO: 24, or a fragment thereof which is at least ten bases long, and

primer Y has the sequence of SEQ ID NO: 25, or a fragment thereof which is at least ten bases long; or

(ii) primer X has the sequence of SEQ ID NO: 26, or a fragment thereof which is at least ten bases long, and

primer Y has the sequence of SEQ ID NO: 27, or a fragment thereof which is at least ten bases long;

and the amplified cytochrome P450 3A5 (CYP3A5) PCR fragment is in between primers X and Y; and

(c) sequencing the amplified fragment obtained in step (b), thereby determining the cytochrome P450 3A5 (CYP3A5) exon 7 genotype of said subject.

Claim 24-25: (canceled)

Attorney Docket No.: 44158/244344 (SJ-0029)
Inventors: Schuetz et al.
Serial No.: 09/974,619
Filing Date: October 10, 2001
Page 31

Claim 26: (currently amended) A method for determining cytochrome P450 3A5 (CYP3A5) genotype of a subject which comprises:

- (a) isolating nucleic acid from said subject;
- (b) making a first and a second PCR primer wherein
 - (i) the first PCR primer is complementary to exon 7 and introduces a base change in the PCR product produced by amplification with the first and second primer adjacent to or near the position corresponding to nucleotide 29 of SEQ ID NO:74 in exon 7, such that a restriction site is generated in the presence of a particular nucleotide at the position corresponding to nucleotide 29 of SEQ ID NO:74 in exon 7; and
 - (ii) the second PCR primer is complementary to a region 3' to the exon 7 nucleotide in the position corresponding to nucleotide 29 of SEQ ID NO:74 in exon 7;
- (c) amplifying the sequence in between the first and the second primers; thereby obtaining an amplified fragment; and
- (d) treating the amplified fragment obtained in step (c) with a restriction enzyme in its corresponding restriction buffer to detect presence or absence of a point mutation at the position corresponding to nucleotide 29 of SEQ ID NO:74 in exon 7, thereby determining the cytochrome P450 3A5 (CYP3A5) genotype of said

Attorney Docket No.: 44158/244344 (SJ-0029)
Inventors: Schuetz et al.
Serial No.: 09/974,619
Filing Date: October 10, 2001
Page 32

subject.

Claim 27: (currently amended) The method of claim 26 wherein the first primer introduces a *Tru9I/MseI* restriction site in the presence of an A nucleotide at the position corresponding to nucleotide 29 of SEQ ID NO:74 in exon 7, and the second primer has the sequence selected from SEQ ID NO:32 and SEQ ID NO:16, or a fragment thereof which is at least ten bases long.

Claim 28: (currently amended) The method of claim 26 wherein the first primer has the sequence ~~corresponding to~~ of SEQ ID NO: 34, or a fragment thereof which is at least ten bases long, and the second primer has the sequence ~~corresponding to~~ of SEQ ID NO: 32, or a fragment thereof which is at least ten bases long.

Claim 29 (currently amended) The method of claim 26 wherein the first primer has the sequence ~~corresponding to~~ of SEQ ID NO:34, or a fragment thereof which is at least ten bases long, and second primer has the sequence ~~corresponding to~~ of SEQ ID NO:16, or a fragment thereof which is at least ten bases long.

Claim 30: (currently amended) A method for determining

Attorney Docket No.: 44158/244344 (SJ-0029)
Inventors: Schuetz et al.
Serial No.: 09/974,619
Filing Date: October 10, 2001
Page 33

cytochrome P450 3A5 (CYP3A5) exon 7 genotype of a subject which comprises:

- (a) isolating nucleic acid from said subject;
- (b) amplifying a cytochrome P450 3A5 (CYP3A5) PCR fragment from said nucleic acid using a first set of primers, wherein said first set of primers contains primer X and primer Y; wherein
 - (i) the X primer is complementary to a region 5' to the position corresponding to nucleotide 29 of SEQ ID NO:74 in exon 7; and
 - (ii) the Y primer is complementary to a region 3' to the position corresponding to nucleotide 29 of SEQ ID NO:74 in exon 7;and the amplified cytochrome P450 3A5 (CYP3A5) PCR fragment is in between primers X and Y, thereby obtaining ~~an~~ a first round amplified fragment;
- (c) amplifying the first round amplified fragment of step (b) using a second set of primers, wherein said second set of primers contains primer Z and primer W, wherein
 - (i) primer Z is complementary to exon 7 and introduces a base change in ~~a~~ the PCR product produced by amplification with the second set of primers adjacent to or near the position corresponding to nucleotide 29 of SEQ ID NO:74 in exon 7, such

Attorney Docket No.: 44158/244344 (SJ-0029)
Inventors: Schuetz et al.
Serial No.: 09/974,619
Filing Date: October 10, 2001
Page 34

that a restriction site is generated in the presence of a particular mutation at the position corresponding to nucleotide 29 of SEQ ID NO:74 in exon 7; and

(ii) primer W is complementary to a region 3' to exon 7;

and the amplified sequence is in between primers Z and W; and

(d) treating the amplified fragment obtained in step (c) with a restriction enzyme in its corresponding restriction buffer to detect presence or absence of a point mutation at the position corresponding to nucleotide 29 of SEQ ID NO:74 in exon 7, thereby determining the cytochrome P450 3A5 (CYP3A5) genotype of said subject.

Claim 31: (currently amended) The method of claim 30 wherein primer X has the sequence ~~corresponding to~~ of SEQ ID NO:30, or a fragment thereof which is at least ten bases long; primer Y has the sequence of SEQ ID NO: 16, or a fragment thereof which is at least ten bases long; primer Z introduces a *Tru9I/MseI* restriction site in the presence of an A nucleotide at the position corresponding to nucleotide 29 of SEQ ID NO:74 in exon 7; and primer W has the sequence selected from SEQ ID NO:32 and SEQ ID NO:16, or a fragment thereof which is at least ten bases

Attorney Docket No.: 44158/244344 (SJ-0029)
Inventors: Schuetz et al.
Serial No.: 09/974,619
Filing Date: October 10, 2001
Page 35

long.

Claim 32: (currently amended) The method of claim 31 wherein primer Z has the sequence ~~corresponding to~~ of SEQ ID NO:34, or a fragment thereof which is at least ten bases long.

Claim 33-38: (canceled)